NSLS Users Determine the Structure of a Protein "Gateway" in Cells

Scientists working at the Brookhaven National Laboratory have determined the structure of a tiny "gateway" protein on the surface of cells that allows other proteins to exit the cell. This structure, determined at the National Synchrotron Light Source (NSLS), is a significant scientific achievement that may provide insight into the complex mechanisms of several essential cellular functions. The structure is described in the January 1, 2004 issue of *Nature*.

These pore-like gateways are situated within the cell's protective, greasy outer "skin," called the membrane. The membrane contains thousands of pores, known as channels, which allow only certain substances, such as nutrients and energy sources, to enter and exit the cell. There are many types of channels, and each type is constructed to permit the passage of specific molecules.

The channel studied in this research is a protein-conducting channel. It permits "secretory proteins" to leave the cell by passing straight through the membrane and allows "membrane proteins" to integrate into the membrane so that parts of the protein appear on the outer side of the membrane. This passage system, called protein translocation, allows the proteins to perform many of an organism's essential functions: For example, secretory proteins, such as digestive enzymes and antibodies, leave the cell to help digest food and fight off disease, respectively, while membrane proteins are needed at the cell surface to fire off nerve signals or trigger the immune system.

Understanding precisely how the proteins traverse the channel – a question many scientists would like to answer – first requires determining the channel's intricate molecular structure. This task was successfully completed at the NSLS by a collaboration of scientists from Harvard Medical School, the Max Planck Institute of Biophysics in Frankfurt, Germany, and the University of Luebeck in Luebeck, Germany.

"The structure tells us much more than we ever hoped to be able to get out of it," said Tom Rapoport, a cell biologist at Harvard and the team's lead scientist. "We can now propose mechanisms for how the channel is opened, how other molecules are prevented from crossing the membrane, and other processes. The structure initiates a new era of more directed research into the mechanism of protein translocation."

At the NSLS, Rapoport and his colleagues slowly imaged and pieced together the components of the channel's overall structure. The final product is a huge step in the field of cell biology: a channel imaged to a degree of detail that had previously only been achieved for channels that transport much smaller molecules, such as water and ions.

The channel is shaped like an hourglass, but behaves quite differently, thanks to certain features. For example, the bottom region of the channel is separated from the top region by a molecular "plug" that swings open to allow the passage of outbound proteins and forms a barrier between the inside of the cell and the external aqueous environment. At the narrow "neck" connecting the top and bottom parts of the hourglass, the channel is lined by a gasket-like "pore ring" that forms a seal around exiting proteins. This keeps smaller molecules from sneaking in or out of the cell.

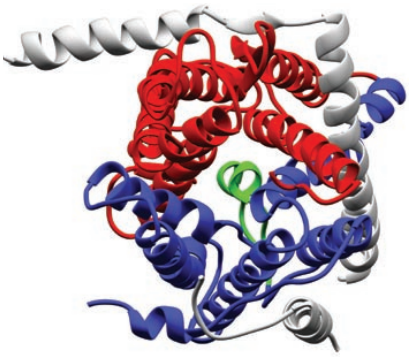
From left, William Clemons, Jr., Tom Rapoport, and Bert van den Berg

The channel also consists of a component that acts somewhat like two molecular "hands" clasped around a cup. By tightening or releasing their grip, the hands narrow or widen the pore, allowing it to admit a wide variety of proteins and shut away unwanted molecules. The hands can also unclasp at the point where their "fingertips" meet. This release mechanism permits proteins to slip sideways out of the channel and become integrated into the membrane.

Assembling this biological puzzle was a great challenge for the researchers. "This is the culmination of 25 years of work on the mechanism of protein translocation, and so it's rewarding to finally

see its three-dimensional structure," Rapoport said. "This is a dream for me."

The first step in that long process, selecting the organism that would produce the best sample to study, was a major hurdle. "We had to go through 10 different bacterial species," said Rapoport. "Finally, we found that a complex derived from an archaebacterium, a primitive organism, was the most stable. Altogether, it took us about five years."



A top view of the protein-conducting channel, looking down into it. The channel's "plug" is shown in green.

The researchers trapped the protein channel in a crystal form, a process that is like taking a three-dimensional "snapshot." Then, working at NSLS beamline X25 and Argonne National Laboratory, they studied the crystals using a technique known as x-ray crystallography: They placed the crystals in the path of an x-ray beam and recorded the pattern the rays created as they scattered and bounced off its molecules. Then, they used a computer to analyze the pattern and determine the structure of the protein in the crystal.

"The next big goal is to crystallize the channel in action," Rapoport concluded.

The other members of the research collaboration are Bert van den Berg, William Clemons, Jr., Yorgo Modis, and Stephen Harrison, all from Harvard Medical School; Ian Collinson, from the Max Planck Institute of Biophysics; and Enno Hartmann, from the University of Luebeck.

—Laura Mgrdichian